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(54) Title: GENETIC TRANSFORMATION USING A PARP INHIBITOR

(57) Abstract

The invention concerns a process for producing transgenic eucaryotic cells, particularly plants, which comprises: contacting a culture of untransformed cells with an inhibitor of poly-(ADP-ribose) polymerase for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce their metabolism. The untransformed cells are then contacted with foreign DNA comprising at least one gene of interest under conditions in which the foreign DNA is taken up by the untransformed cells and the gene of interest is stably integrated in the nuclear genome of the untransformed cells to produce the transgenic cells. Optionally, the transgenic cells are recovered from the culture. Preferably, the inhibitor is niacinamide, preferably at a concentration of about 200 mg/l to 500 mg/l and the untransformed cells are cultured in a medium containing the inhibitor for a period of time of approximately 3 to 14 days prior to the contacting with the foreign DNA. The invention also relates to a plant having in the nuclear genome of its cells foreign DNA integrated only in the regions of the nuclear genome that are transcriptionally active in cells of the plant when the cells are treated with an effective amount of a PARP inhibitor for a period of time sufficient to reduce cell metabolism to a state where gene expression is essentially limited to genes expressed irrespective of the differentiated or physiological condition of the cell.

GENETIC TRANSFORMATION USING A PARP INHIBITOR

This invention is related to tissue culture of eucaryotic cells and improved techniques to obtain genetically transformed eucaryotic cells and organisms, such as transgenic plant cells or plants, by lowering the stress reaction of cultured eucaryotic cells prior to contacting the cells with foreign DNA, particularly by specific inhibition of poly-(ADP-ribose) polymerase.

Background to the invention

Over the years many techniques for the genetic transformation of higher organisms (animals and plants) have been developed. In these techniques it is the ultimate goal to obtain a transgenic organism, e.g. a plant, in which all cells contain a foreign DNA comprising a gene of interest (the so-called transgene) stably integrated in their genome, particularly their nuclear genome.

Transformation is a complex process which always involves the contacting of starting cells with a DNA, usually a DNA comprising foreign gene(s) of interest. The contacting of the cells with the DNA is carried out under conditions that promote the uptake of the DNA by the cells and the integration of the DNA, including the gene(s) of interest into the genome of the cell.

Starting cells for transformation are usually cells that have been cultured in vitro for some time. After contacting the cells with the DNA, the transformed cells generally need to be cultured in vitro for a certain period in order to separate the transformed cells from the non-transformed cells and, in the case of plants, to regenerate transformed plants from the transformed cells. Indeed, complete plants can be regenerated from individual transformed cells thus ensuring that all cells of the regenerated plant will contain the transgene.

number of the introduced DNA at a given locus. It has been suggested that some of the variability in expression of introduced genes in transgenic plants is a consequence of "position effects" caused by influences of adjacent plant genomic DNA. Other factors that could contribute to the variability in expression are physiological variability of the plant material, differences in the number of independent T-DNA loci in different transformants or the inhibitory effects of certain T-DNA structures on gene expression. Between-transformant variability in expression has been observed for the majority of introduced genes in transgenic plants. The variability in expression of many introduced genes in independent transgenic plants necessitates large numbers of transgenic plants to be assayed to accurately quantitate the expression of the gene. It would be of great importance if the amount of between-transformant variability could be reduced (Dean et al, 1988, NAR 16:9267-9283).

If the transgene is under the control of a tissue-specific promoter, with the expectation that it will be expressed in selected tissues of the transformed organisms, the position effects can lead, at least in some transformants, to loss of specificity of the promoter and expression of the transgene in undesired tissues, e.g. in tissue cultured in vitro.

Factors that are known to influence the efficiency and quality of the genetic transformation process are the method of DNA delivery, specific tissue culture conditions, the physiological and metabolic state of the target cells etc. Direct gene transfer methods for instance are generally known to result in transformed organisms with a high copy number of the transgene.
Many of these factors are not under the control of man.

used to influence the metabolic state of cells which are targeted for transformation (or which are being transformed) to increase the efficiency and/or quality of transformation.

In mammalians, PARP is a monomeric nuclear Zn-finger protein of about 116 kD that is closely associated with nuclear DNA, particularly with actively transcribed euchromatic regions (Shah et al, 1995, Anal.Biochem. 227:1-13). The protein is normally an inactive enzyme but is known to be activated by nicked or otherwise damaged DNA. Active PARP transfers the ADP-ribose moiety of NAD+ to various nuclear proteins to synthesize a polymer of ADP-ribose bound to these proteins which include PARP itself, polymerases, histones, endonuclease etc. The proteins on which such a ADP-ribose polymer is synthesized become biologically inactive (de Murcia et al, 1994, TIBS 19:172-176; Cleaver et al, 1991, Mutation Res. 257:1-18).

The biological function of PARP is largely unknown but the enzyme has been implicated in :

- enhancement of DNA repair (Satoh et al, 1992, Nature 356:356-358; Satoh et al, 1993, J.Biol.Chem. 268:5480-5487),
- recombination events : in general inhibition of PARP is observed to inhibit illegitimate recombination and to increase intrachromosomal recombination but it does apparently not affect extrachromosomal recombination (Farzaneh et al, 1988, NAR 16:11319-11326; Waldman and Waldman, 1990, NAR 18:5981-5988; Waldman and Waldman, 1991, NAR 19:5943-5947),
- regulation of gene expression : inhibition of PARP is observed to decrease gene expression (Girod et al, 1991,Plant Cell, Tissue and Organ Culture 25:1-12);
- reducing the amount of available NAD+ (and by consequence its precursor ATP) : this results in a general slowing down of cell metabolism (Lazebnik

time") at which the cells are contacted with foreign DNA comprising one or more genes of interest. However, depending on the purpose, the PARP inhibitor may also be added to the culture medium during and/or after the contacting time or even solely after the contacting time.

In one aspect of this invention treatment of cultured cells, tissues or organs with PARP inhibitors may be used to increase the quality of transformation as measured by the copy number of the transgene and by variation in transgene expression (quality and quantity) in the transformed cells and in organisms obtained from the transformed cells.

In many conventional procedures for genetic transformation of eucaryotic cells, particularly plant cells, cultured cells, tissues or organs will be used as starting material and cells in such cultures will be contacted with foreign DNA comprising at least one gene of interest (i.e. the transgene) under conditions that will promote the uptake of the foreign DNA in the cells and the ultimate integration of the foreign DNA into the genome of the cells.

In one embodiment of the invention it is preferred that a PARP inhibitor is added to the culture medium for a period of at least 2-3 days, preferably at least about 3 days, prior to contacting the cells with the foreign DNA. The exact period in which the cultured cells are incubated in PARP inhibitor containing medium is believed not to be critical but should probably not exceed 4 weeks. It appears that 2-14 days, particularly 3-10 days, is an optimal period and best results were obtained with an incubation period of approximately 4 to 5 days prior to the contacting time. Generally it is believed that 4 days is a useful period for the PARP inhibitor to be added to the culture medium prior to the contacting time.

The concentration of the PARP inhibitor in the medium is also believed to have an effect on the inhibition of PARP, which varies depending on the nature of the cells (species, tissue explant, general culture conditions, etc.) However,

For other PARP inhibitors optimal concentrations can be easily established by experimentation in accordance with this invention.

During transformation it is not known whether the integration of the DNA into the genome of the cell occurs immediately after uptake of DNA by the cell. It may very well be that the foreign DNA exists as free DNA within the cell for a certain period after the contacting time. Therefore cultured cells may be further incubated in medium containing a PARP inhibitor during and, for a limited period of time after, contacting the cells with the foreign DNA. Again the length of the incubation period is not critical but is preferably 2-10 days, particularly approximately 4 days. It is preferred that the inhibitor concentration of the PARP inhibitor in the culture medium after the contacting time should be below 2 mM, between 0.8 and 1 mM. If the cells that are to be transformed are not obtained from a cell or tissue culture (e.g. when intact tissue of an organism is contacted directly with DNA, as for example described in WO 92/09696) the PARP inhibitor may still be applied to the target cells prior to the contacting time but the addition of the PARP inhibitor to the culture of the transformed cells during or after the contacting time is preferred.

As indicated above, PARP inhibitor treatment of cultured cells for at least 2-3 days increases the quality of transformation. Indeed the number of copies of the foreign DNA is expected to be generally lower and variation in expression profile (level - i.e. the quantity - of expression as well as spatial and time distribution - i.e. the quality - of expression in the transgenic organism) of the gene(s) of interest in the foreign DNA, due to position effects, is decreased. However, at least in this aspect of the invention, the efficiency of transformation can be decreased. The efficiency of transformation as used herein can be measured by the number of transformed cells (or transgenic organisms grown from individual transformed cells) that are recovered under standard experimental conditions (i.e. standardized or normalized with respect to amount

of lesser importance since Agrobacterium mediated transformation, being a biological process, already results in a generally low copy number of the transgene in the transformed plant cells.

In accordance with this invention the addition of PARP inhibitors, such as niacinamide, to the culture medium of eucaryotic cells, can be used in combination with any known transformation procedure that requires cells, tissues or organs cultured in vitro as starting cells to be contacted with foreign DNA. The process of this invention is thus generally identical to existing conventional transformation methods except for the fact that at some times during the tissue culture of the cells, a PARP inhibitor is added to the culture medium.

The cell of a plant, particularly a plant capable of being infected with Agrobacterium such as most dicotyledonous plants (e.g. Brassica napus) and some monocotyledonous plants, can be transformed using a vector that is a disarmed Ti-plasmid containing the gene(s) of interest and carried by Agrobacterium. This transformation can be carried out using conventional procedures (EP 0,116,718; Deblaere et al, supra; Chang et al, 1994, The Plant Journal 5:551-558). Preferred Ti-plasmid vectors contain the foreign DNA between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example, in EP 0,233,247), pollen mediated transformation (as described, for example, in EP 0,270,356, PCT patent publication "WO" 85/01856, and US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in EP 0,067,553 and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475). Cells of monocotyledonous plants such as the major cereals including corn, rice, wheat, barley, and rye, can be

accordance with this invention. As described above it is believed that in cells treated with a PARP inhibitor for at least 3 days, particularly for at least 4 days, only a limited number of genomic regions will remain transcriptionally active. In this regard the transformed cells, obtained with this process of the invention, will be characterized by having the foreign DNA integrated in a limited number of genomic regions. That the transformed cell or organism was obtained by this process of the invention can thus be easily ascertained by 1) culturing transformed cells or tissues under conditions that are similar as those in which the untransformed cells or tissues were grown or incubated prior to the integration of the foreign DNA in the genome (i.e. incubating in medium containing 250 mg/l niacinamide for 4-5 days prior to the contacting time), and 2) monitoring the expression of at least one transgene in the foreign DNA that is expected to be expressed under normal tissue culture conditions (i.e. a selectable marker gene under the control of a promoter that directs expression in tissue culture). Under the above conditions the transformed cells or tissues of this invention express the relevant transgene in the tissue culture at essentially the same levels whether or not a PARP inhibitor is present in the culture medium. It is thus expected that, for instance after 4-5 days of culturing of the transformed cells in medium containing the PARP inhibitor, mRNA levels are not signicantly decreased, i.e. do not become lower than 75%, preferably not become lower than 90%, when compared to the mRNA levels observed in cells cultured in medium not containing the inhibitor. Indeed, if the relevant transgene is integrated in other regions of the genome (i.e. in regions that are normally not transcriptionally active in cells treated with PARP inhibitor according to this embodiment of the invention), the expression of the relevant transgene is considerably reduced after incubation of the cells in medium containing the PARP inhibitor for at least 3 days, e.g. 4-5 days (i.e. mRNA levels will drop below 75%, particularly below 50%, more particularly below

transformation may be reduced but the average quality of transformation is expected to be significantly improved because of lower copy number of the gene of interest in the genome of the transformed cells and because of reduced position effects i.e. the general integration of the gene of interest in the genomes at locations that minimally affect the transcriptional properties of the promoter of the transgene.

The foreign DNA used in the method of this invention generally also comprises a selectable marker gene the expression of which allows the selection of transformed cells (or organisms) from non-transformed cells (or organisms). Such selectable marker gene generally encodes a protein that confers to the cell resistance to an antibiotic or other chemical compound that is normally toxic for the cells. In plants the selectable marker gene may thus also encode a protein that confers resistance to a herbicide, such as a herbicide comprising a glutamine synthetase inhibitor (e.g. phosphinothricin) as an active ingredient. An example of such genes are genes encoding phosphinothricin acetyl transferase such as the sfr or sfrv genes (EP 242236; EP 242246; De Block et al, 1987 EMBO J 6:2513-2518).

The inventors also found that the initial reaction of cells, particularly cells contacted with PARP inhibitors, is a stress reaction which enhances free radical production by the cell. However, this stress only lasts for a limited period of time after which further contact with the PARP inhibitor causes a decrease in cell metabolism, particularly a decrease in electron flow in the mitochondrial electron transport chain. Therefore, the invention also relates to a new method to assess the agronomical fitness of a population of transformed plants to determine in which lines the plants have a foreign DNA integrated in their genomes in a way that agronomical performance is not or substantially not affected. The assay is based on comparative reaction of transgenic cells and corresponding untransformed controls to stress conditions.

endogenous genes by the transgene(s), or that expression of endogenous genes may be deregulated by sequences in the foreign DNA. As a consequence many transformed lines may not be agronomically useful.

The assay of this invention will for example allow to identify a line (i.e. a group of genetically similar plants) of transformed plants that have the transgene(s) integrated in regions that minimally affect the fitness of the plants, thus avoiding the extensive laboratory, greenhouse and/or field evaluations which are normally required to identify the transformants with the best agronomical properties.

The assay in accordance with this invention essentially comprises the incubation of cells or tissues of transformed plants of a particular transgenic line (e.g. callus, hypocotyl explants, shoots, leaf disks, whole leaves etc.) preferably with a PARP inhibitor (although for some plant species this is not necessary) under a range of conditions which induce the production of a different amount of free radicals in the tissues. An incubation time of approximately one day is normally sufficient to generate the desired amount of free radicals. Appropriate controls, i.e. untransformed tissues obtained from untransformed plants at the same developmental stage and grown in the same conditions as the transformed plant from which the transformed tissue was obtained, are subjected to the same treatment. Preferably the untransformed line is identical to the transgenic line except for the presence of the transgene(s).

For each plant line (control or transformant) it is preferred that a number of plants is assayed.

Useful conditions for the incubation of the untransformed and transformed tissues are those which induce increasing osmotic and salt stress in the incubated cells or tissues. For example a series of buffers with different salt

- spectrophotometric quantification of reduced TTC at 485 nm (optical density OD_{485}; for chlorophyll poor plant material) or 545 nm (OD_{545}; for chlorophyll rich plant material). The O.D. of the control extract is subtracted from the OD of the TTC-reacted extracts. In the above conditions 0.1 mM reduced TTC corresponds to an OD_{485} of 0.214 or OD_{545} of 1.025 (light path 1 cm).
- the reducing capacity of the transformed plant line is compared to that of the control line.

The amount of reduced TTC is determined by the intensity of the cytochromal and alternative respiratory pathways and the radical concentration in the tissues which, in turn are determined by the presence of mutations, the expression of genes affecting the metabolic activity of the plant cells, the developmental stage and the reaction of the tissue to external factors, such as stress factors.

The TTC reducing capacity (as for instance measured by the O.D. at 485 nm) for tissues incubated at high salt concentration (TTC-high) is expressed as the percentage of the TTC reducing capacity of the tissues incubated at a low salt concentration (TTC-low); in other words a TTC-ratio value is calculated as follows:

TTC-ratio = TTC/high.100/TTC.low.

The value of TTC-ratio is a measure of the fitness of a plant line as compared to a control line.

The determination of TTC-low and TTC-high will depend on the sensitivity of the plant species to the applied salt stress. Usually TTC-low will correspond to a salt concentration between 10 and 25 mM K-phosphate, e.g. at 20 mM while TTC-high will correspond to a salt concentration between 50 and 80 mM K-phosphate. The only requirement is that TTC-high should be significantly lower than TTC-low; preferably TTC-high should be lower than 50% of TTC-low, particularly lower than 30% of TTC-low. For instance for Brassica napus, TTC-

The TTC-reducing assay can further be used in a modified way to determine the quality and the fitness of plant material, for example plant material to be used in transformation experiments (i.e. whether particular plant material, e.g. explants, is suitable as starting material). To this end the TTC-reducing assay can be adapted for example in the following way:

1. A sample of the plant material to be tested for its suitability for transformation, is incubated for one day in plant culture medium or a buffer containing 2% sucrose and a K-phosphate concentration ranging between 10 and 80 mM, typically around 25 mM, to which a suitable amount of a PARP inhibitor, such as niacinamide has been added. For niacinamide, a preferred concentration to be used is 250 mg/L, although concentrations as low as 100 mg/L and as high as 1000 mg/L may be used. A comparable control sample of the same plant material is incubated under similar conditions without PARP inhibitor.
2. After one day of incubation the capacity of the plant material incubated with PARP inhibitor and the control plant material to reduce TTC is measured by the procedure described above.

The TTC reducing capacity (as for instance measured by the O.D. at 485 nm) for plant material incubated with PARP inhibitor (TTC-INH) is compared with the TTC reducing capacity of the control plant material incubated without PARP inhibitor (TTC-CON) and a ratio (E) is calculated as follows:

$$E = \text{TTC-INH} / \text{TTC-CON}$$

The value E is a measure of the quality and fitness of the plant material, for example explants to be transformed. It is believed that those tissues, wherein the E value is larger than or equals 1, are healthy tissues, which are particularly suitable as starting material for transformation.

The modified TTC-procedure thus allows to select those types of (cultured) plant material especially appropriate for use in a transformation procedure,

peculiar aspect of fitness of cells, tissue, explant or organism. For instance, it is possible to apply a type of stress different from osmotic or salt stress, such as stress brought about by extreme temperatures, by sublethal treatment with chemicals (e.g. herbicides, heavy metals) or by irradiation with UV. Furthermore, other types of PARP inhibitors, as mentioned before may be used, within the indicated concentration ranges. Although it is believed that for the purpose of the assays defined here, TTC is the most suited substrate, other indicator molecules ,such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium) can be used to measure the electron flow in the mitochondrial electron transport chain downstream of the "ubiquinone pool".

Unless otherwise indicated all experimental procedures for manipulating recombinant DNA were carried out by the standardized procedures described in Sambrook et al., 1989, "Molecular Cloning: a Laboratory Manual", Cold Spring Harbor Laboratory, and Ausubel et al, 1994, "Current Protocols in Molecular Biology", John Wiley & Sons.

The polymerase chain reactions ("PCR") were used to clone and/or amplify DNA fragments. PCR with overlap extension was used in order to construct chimeric genes (Horton et al, 1989, Gene 77:61-68; Ho et al, 1989, Gene 77:51-59).

All PCR reactions were performed under conventional conditions using the Vent™ polymerase (Cat. No. 254L - Biolabs New England, Beverley, MA 01915, U.S.A.) isolated from Thermococcus litoralis (Neuner et al., 1990, Arch.Microbiol. 153:205-207). Oligonucleotides were designed according to known rules as outlined for example by Kramer and Fritz (1968, Methods in Enzymology 154:350), and synthesized by the phosphoramidite method (Beaucage and Caruthers, 1981, Tetrahedron Letters 22:1859) on an applied Biosystems 380A DNA synthesizer (Applied Biosystems B.V., Maarssen,

Similar observations were made when Brassica napus hypocotyl explants were cultured on A5 medium (see Example 3) containing 250 mg/l niacinamide. It was also observed that, in Brassica napus tissue cultured on medium containing niacinamide, no anthocyanin was produced; normally anthocyanin in tissue culture is produced in stress conditions. In addition it was observed that after 4-5 days of incubation of the plant tissue with niacinamide, the concentrations of hydroxyl free radical and dehydroascorbate in the explants were drastically decreased.

It was also observed that, after a 4 day incubation in niacinamide containing medium, the percentage of cultured cells that were in G2 phase of the cell cycle was considerably increased (up to 45 % of all cells in the culture).

The above observations are interpreted as indicating that treating cultured cells with a PARP inhibitor for about 4-5 days generally results in :

1) a significant reduction of the response of the cultured cells to stress as measured for instance by free radical and/or anthocyanin production , and

2) a reduction of the general metabolism of the cultured cells to a very basic level as indicated by the fact that the tissue growth was slowed down, and the TTC reducing capacity was decreased while the tissue remained viable.

It is inferred that under these conditions many genes in cells (e.g. cultured cells) that would normally be switched on in response to stress (such as during transformation conditions) will in fact no longer be induced. It is expected that in such cells which only display a very basic metabolism, mainly general "housekeeping genes", i.e. genes that are expressed in any cell irrespective of its differentiated state or metabolic or physiological condition, are expressed.

As it is believed that foreign DNA is preferably inserted in portions of the genome that are transcriptionally active it follows that treatment with PARP inhibitors will condition eucaryotic cells to incorporate any foreign DNA

only 0.5 mg/l $CuSO_4.5H_20$ and 1 mg/l 2,4-D), and incubated for 3 weeks at 24-25°C in the light (approx. 20 mEinsteins/s/m^2 (with a photoperiod of 16 hours light and 8 hours dark).

About 2 weeks prior to bombardment the calli were cleaned up by removal of non-morphogenic (i.e. the nonembryogenic and nonmeristematic) parts and were subcultured on W2 medium.

For bombardment the calli were divided into small pieces with an average maximum diameter of about 2-3 mm. These pieces were placed at the center of a 9 cm Petridish containing W2 medium in a circle with a diameter of approx. 0.5 cm. When required niacinamide (250 mg/l) was added to the W2 medium and the tissue pieces were maintained under these conditions for 4 days after they were bombarded.

Bombardment was carried out using the Biolistic PDS-1000/He apparatus (Bio-Rad). Preparation of the microcarriers (0.4-1.2m) and the coating of the microcarriers with DNA was essentially carried out according to the manufacturer's instructions. The Petridishes containing the calli were placed at level 2 of the apparatus and the bombardment was done at 1550 psi.

For the transformation experiments the following plasmid DNA was used.

- plasmid pVE136, the sequence of which is given in SEQ ID No 4. This plasmid contains the following chimeric genes:
  - P35S-bar-3'nos
  - PCA55-barnase-3'nos

  in which P35S is the 35S promoter of the Cauliflower Mosaic virus, bar is a DNA encoding phosphinothricin acetyltransferase (EP 242236), 3'nos is the 3' untranslated end of the Agrobacterium T-DNA nopaline synthase gene, PCA55 is a stamen-specific promoter from corn gene CA55 (WO

$CuSO_4.5H_20$ and 0.5 mg/l 2,4-D). Calli were subcultivated every two weeks at which time the nonmorphogenic parts of the calli were removed. When the calli started to form shoots they were transferred to W5 medium (W1 medium with half concentrated MS medium and only 0.5 mg/l $CuSO_4.5H_20$ and without acetylsalicylic acid and 2,4-D, but supplemented with 50 mg/l myo-inositol, 0.25 mg/l pyridoxine.HCl and 0.25 mg/l nicotinic acid) containing 2.5 mg/l PPT. For the rest of the procedure temperature was maintained at a maximum of 24°C. The calli were subcultivated every 3-4 weeks. Once the shoots started to elongate and small roots started to form, the whole calli (or if possible individual shoots) were transferred to 1 liter vessels with W6 medium (half-concentrated MS medium supplemented with 1.5% sucrose, 50 mg/l myo-inositol, 0.25 mg/l pyridoxine.HCl, 0.25 mg/l nicotinic acid, 0.5 mg/l thiamine.HCl, 0.7% agar (Difco) pH 5.8 and 0.5 mg/l $CuSO_4.5H_2O$) containing 2.5 mg/l PPT. Once the shoots and roots had grown out, individual shoots were separated from each other and transferred to 1 l vessels containing W6 medium with 2.5 mg/l PPT. Well developed shoots are tested for PPT resistance by means of the TLC assay (De Block et al, 1987, EMBO 6:2513-2518) or by direct assay of ammonium production in the tissue (see e.g. De Block et al, 1995, Planta 197: 619-626). Transformed shoots were finally transferred to the greenhouse into soil.

For analysis of the results the transformed plants could be subdivided according to the niacinamide treatment of the parent calli during tissue culture. Thus the following groups were distinguished:

Group	Niacinamide treatment
None	No treatment
Before 100	100 mg/l niacinamide for four days prior to bombardment
Before 250	250 mg/l niacinamide for four days prior to bombardment

Results of wheat transformation experiments

Table 1 :

Plasmid pTS172				
Treatment	Nr of bombarded calli	Nr of PPT-resistant calli recovered	Nr of PPT resistant plants recovered	Nr of MS plants recovered
None	60	30	1[a)]	0
Before 250	125	30	3	3[b)]

a) This plant proved to be fertile and to be transformed only with the chimeric bar gene

b) The obtained plants looked healthy and tillered vigorously

Table 3:

Plasmid pVE136			
Treatment	Nr of bombarded calli	Nr of PPT resistant plants recovered	Nr of MS plants recovered
None	200	1	0
Before 100	800	8[a)]	8

a) The obtained plants looked healthy and tillered vigorously

Plasmid pTHW107 is a vector carrying a T-DNA comprising the following chimeric genes :

- PTA29-barnase-3'g7
- PSSU-bar-3'nos

in which PTA29 is the promoter of the TA29 gene of tobacco (EP 344029) and PSSU is the promoter of the gene of Arabidopsis thaliana encoding the small subunit of Rubisco. The complete sequence of the T-DNA of pTHW107 is presented in SEQ ID No 1.

Where required niacinamide (250 mg/l) was added to the media for the last 4 days prior to infection with Agrobacterium. Plants regenerated from transformed calli obtained on niacinamide cultured cells were observed to have a low copy number as well as to display less variation in the expression profile of the transgenes (results summarized in Table 4). Five plants regenerated from the calli obtained by transformation including niacinamide and five plants regenerated from the calli obtained by conventional transformation without niacinamide inclusion, were analyzed by Southern hybridization to determine the copy number of the transgenes, and were further analyzed for reproductive phenotype. In the non-treated group, a substantial number of regenerated plants proved not to have a transgene integrated in their nuclear DNA.

Example 4: Agrobacterium-mediated transformation of oilseed rape using niacinamide in the culture medium.

Hypocotyl explants of Brassica napus were obtained as described in Example 3. Four groups of 200 hypocotyl explants each, were either not treated with niacinamide (indicated in table 4 as NONE), treated with 250 mg/l niacinamide for 1 day prior to infection with Agrobacterium (BEFORE), treated for 2 days during the infection with 250 mg/l niacinamide (DURING), or treated for 1 day after the Agrobacterium infection with 250 mg/l niacinamide (AFTER).

All hypocotyl explants were infected with Agrobacterium tumefaciens strain C58C1Rif carrying T-DNA vector pTHW142 and a helper Ti-plasmid pMP90 (Koncz and Shell, 1986 supra)(or a derivative thereof).

Plasmid pTHW142 is a vector carrying a T-DNA comprising the following chimeric genes:

- PSSU-bar-3'g7
- p35S-uidA-3'35S

In which uidA is a DNA encoding b-glucuronidase (Jefferson et al., 1986, Proc. Natl. Acad. Sci. USA 83, 8447-8451) and 3' 35S is the 3' untranslated end of the cauliflower mosaic virus 35S transcript.

The complete sequence of the T-DNA of pTHW142 is presented in SEQ ID No 5.

After the Agrobacterium infection, hypocotyl explants were transferred to selection medium A5, and if appropriate to A5 medium containing 250 mg/l niacinamide. The hypocotyl explants that were placed on medium containing niacinamide were transferred after 1 day to niacinamide-free selection medium A5. After 5 weeks on selective medium the number of transformed calli was scored. b-glucuronidase expression was verified in the obtained calli using established protocols (Jefferson et al.,1986). The results are summarized in

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PLANT GENETIC SYSTEMS N.V.
(B) STREET: Plateaustraat 22
(C) CITY: Ghent
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): 9000
(G) TELEPHONE: 32 9 235 84 58
(H) TELEFAX: 32 9 223 19 23
(I) TELEX: 11.361 Pgsgen

(ii) TITLE OF INVENTION: Genetic Transformation using a PARP inhibitor

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4946 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T-DNA of plasmid pTHW107

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (1..25)
(D) OTHER INFORMATION:/label= RB
/note= "T-DNA right border"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (97..330)
(D) OTHER INFORMATION:/label= 3'g7
/note= "3' untranslated region containing the polyadenylation signal of gene 7 of Agrobacterium T-DNA "

GGGCGGTACC GGCAGGCTGA AGTCCAGCTG CCAGAAACCC ACGTCATGCC AGTTCCCGTG 420

CTTGAAGCCG GCCGCCCGCA GCATGCCGCG GGGGGCATAT CCGAGCGCCT CGTGCATGCG 480

CACGCTCGGG TCGTTGGGCA GCCCGATGAC AGCGACCACG CTCTTGAAGC CCTGTGCCTC 540

CAGGGACTTC AGCAGGTGGG TGTAGAGCGT GGAGCCCAGT CCCGTCCGCT GGTGGCGGGG 600

GGAGACGTAC ACGGTCGACT CGGCCGTCCA GTCGTAGGCG TTGCGTGCCT TCCAGGGGCC 660

CGCGTAGGCG ATGCCGGCGA CCTCGCCGTC CACCTCGGCG ACGAGCCAGG GATAGCGCTC 720

CCGCAGACGG ACGAGGTCGT CCGTCCACTC CTGCGGTTCC TGCGGCTCGG TACGGAAGTT 780

GACCGTGCTT GTCTCGATGT AGTGGTTGAC GATGGTGCAG ACCGCCGGCA TGTCCGCCTC 840

GGTGGCACGG CGGATGTCGG CCGGGCGTCG TTCTGGGTCC ATTGTTCTTC TTTACTCTTT 900

GTGTGACTGA GGTTTGGTCT AGTGCTTTGG TCATCTATAT ATAATGATAA CAACAATGAG 960

AACAAGCTTT GGAGTGATCG GAGGGTCTAG GATACATGAG ATTCAAGTGG ACTAGGATCT 1020

ACACCGTTGG ATTTTGAGTG TGGATATGTG TGAGGTTAAT TTTACTTGGT AACGGCCACA 1080

AAGGCCTAAG GAGAGGTGTT GAGACCCTTA TCGGCTTGAA CCGCTGGAAT AATGCCACGT 1140

GGAAGATAAT TCCATGAATC TTATCGTTAT CTATGAGTGA AATTGTGTGA TGGTGGAGTG 1200

GTGCTTGCTC ATTTTACTTG CCTGGTGGAC TTGGCCCTTT CCTTATGGGG AATTTATATT 1260

TTACTTACTA TAGAGCTTTC ATACCTTTTT TTTACCTTGG ATTTAGTTAA TATATAATGG 1320

TATGATTCAT GAATAAAAAT GGGAAATTTT TGAATTTGTA CTGCTAAATG CATAAGATTA 1380

GGTGAAACTG TGGAATATAT ATTTTTTTCA TTTAAAAGCA AAATTTGCCT TTTACTAGAA 1440

TTATAAATAT AGAAAAATAT ATAACATTCA AATAAAAATG AAAATAAGAA CTTTCAAAAA 1500

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TACAACAAGT CATAAGCCCA ACAAAGTTAG CACGTCTAAA TAAACTAAAG AGTCCACGAA 1620

AATATTACAA ATCATAAGCC CAACAAAGTT ATTGATCAAA AAAAAAAAAC GCCCAACAAA 1680

GCTAAACAAA GTCCAAAAAA AACTTCTCAA GTCTCCATCT TCCTTTATGA ACATTGAAAA 1740

CTATACACAA AACAAGTCAG ATAAATCTCT TTCTGGGCCT GTCTTCCCAA CCTCCTACAT 1800

CACTTCCCTA TCGGATTGAA TGTTTTACTT GTACCTTTTC CGTTGCAATG ATATTGATAG 1860

TATGTTTGTG AAAACTAATA GGGTTAACAA TCGAAGTCAT GGAATATGGA TTTGGTCCAA 1920

GATTTTCCGA GAGCTTTCTA GTAGAAAGCC CATCACCAGA AATTTACTAG TAAAATAAAT 1980

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TCAATTGTCC CTTCTTGTTT GGCACTATAT TCAATCTGTT AATGCAAATT ATCCAGTTAT 3900

ACTTAGCTAG ATATCCAATT TTGAATAAAA ATAGCTCTTG ATTAGTAAAC CGGATAGTGA 3960

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CAGATTGTTA CATGGAAAAC AAAAAGTCCT CTGATAGAAG TCGCAAAGTA TCACAATTTT 4140

CTATCGAGAG ATAGATTGAA AGAAGTGCAG GGAAGCGGTT AACTGGAACA TAACACAATG 4200

TCTAAATTAA TTGCATTCGC TAACCAAAAA GTGTATTACT CTCTCCGGTC CACAATAAGT 4260

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AAATGTGAAT TTCTTAATCT GTGTGAAAAC AACCAAAAAA TCACTTATTG TGGACCGGAG 4500

AAAGTATATA AATATATATT TGGAAGCGAC TAAAAATAAA CTTTTCTCAT ATTATACGAA 4560

CCTAAAAACA GCATATGGTA GTTTCTAGGG AATCTAAATC ACTAAAATTA ATAAAAGAAG 4620

CAACAAGTAT CAATACATAT GATTTACACC GTCAAACACG AAATTCGTAA ATATTTAATA 4680

TAATAAAGAA TTAATCCAAA TAGCCTCCCA CCCTATAACT TAAACTAAAA ATAACCAGCG 4740

AATGTATATT ATATGCATAA TTTATATATT AAATGTGTAT AATCATGTAT AATCAATGTA 4800

TAATCTATGT ATATGGTTAG AAAAAGTAAA CAATTAATAT AGCCGGCTAT TTGTGTAAAA 4860

ATCCCTAATA TAATCGCGAC GGATCCCCGG GAATTCCGGG GAAGCTTAGA TCCATGGAGC 4920

CATTTACAAT TGAATATATC CTGCCG 4946

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6548 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA 240

AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA TCGAACTGGA TCTCAACAGC 300

GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA 360

GTTCTGCTAT GTGGCGCGGT ATTATCCCGT ATTGACGCCG GGCAAGAGCA ACTCGGTCGC 420

CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 480

ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT 540

GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC TTTTTTGCAC 600

AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA 660

CCAAACGACG AGCGTGACAC CACGATGCCT GTAGCAATGG CAACAACGTT GCGCAAACTA 720

TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 780

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT 840

AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG GCCAGATGGT 900

AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA 960

AATAGACAGA TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA 1020

GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG 1080

GTGAAGATCC TTTTTGGCTC GAGTCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC 1140

CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG 1200

CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG 1260

GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA 1320

AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG 1380

CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG 1440

TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA 1500

ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC 1560

CTACAGCGTG AGCATTGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT 1620

CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC 1680

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CAACTAACTG TTTCCGAATT TCAGGGCCCC TCCAAGGATC CCAGAAATCA TCATCTCTGA 3840

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TCTTTACGAC ATTGCATGTG GAAAGGATCT GAAGAGATTT CTCCTGGTAC ATAATAATCT 3960

ACTCCTTTGC TACGTTAATA AGAGATGTAA AAACATGCAA CAGTTCCAGT GCCAACATTG 4020

TCCAAGGATT GTGCAATTCT TTCTGGAGCG CTAAAATTGA CCAGATTAGA CGCATCAGAA 4080

TATTGAATTG CAGAGTTAGC CAATAATCCT CATAATGTTA ATGTGCTATT GTTGTTCACT 4140

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TATTGGTATT GTCGGCGATT GGAAGTTCTT GCAGCTTGAC AAGTCTACTA TATATTGGTA 4260

GGTATTCCAG ATAAATATTA AATTTTAATA AAACAATCAC ACAGAAGGAT CTGCGGCCGC 4320

TAGCCTAGGC CCGGGCCCAC AAAAATCTGA GCTTAACAGC ACAGTTGCTC CTCTCAGAGC 4380

AGAATCGGGT ATTCAACACC CTCATATCAA CTACTACGTT GTGTATAACG GTCCACATGC 4440

CGGTATATAC GATGACTGGG GTTGTACAAA GGCGGCAACA AACGGCGTTC CCGGAGTTGC 4500

ACACAAGAAA TTTGCCACTA TTACAGAGGC AAGAGCAGCA GCTGACGCGT ACACAACAAG 4560

TCAGCAAACA GACAGGTTGA ACTTCATCCC CAAAGGAGAA GCTCAACTCA AGCCCAAGAG 4620

CTTTGCTAAG GCCCTAACAA GCCCACCAAA GCAAAAAGCC CACTGGCTCA CGCTAGGAAC 4680

CAAAAGGCCC AGCAGTGATC CAGCCCCAAA AGAGATCTCC TTTGCCCCGG AGATTACAAT 4740

GGACGATTTC CTCTATCTTT ACGATCTAGG AAGGAAGTTC GAAGGTGAAG GTGACGACAC 4800

TATGTTCACC ACTGATAATG AGAAGGTTAG CCTCTTCAAT TTCAGAAAGA ATGCTGACCC 4860

ACAGATGGTT AGAGAGGCCT ACGCAGCAGG TCTCATCAAG ACGATCTACC CGAGTAACAA 4920

TCTCCAGGAG ATCAAATACC TTCCCAAGAA GGTTAAAGAT GCAGTCAAAA GATTCAGGAC 4980

TAATTGCATC AAGAACACAG AGAAAGACAT ATTTCTCAAG ATCAGAAGTA CTATTCCAGT 5040

ATGGACGATT CAAGGCTTGC TTCATAAACC AAGGCAAGTA ATAGAGATTG GAGTCTCTAA 5100

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T72 promoter region

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (1..1601)
(D) OTHER INFORMATION:/label= PT72
/note= "promoter region of T72 gene of rice"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGCCGTGAGT GTCTTCTGCC GCCGAGGGGC TCTCGCTCGT CGTCGATGCC TGCACGGTGC 60

GTGCGTGTGT GTCGTGGTGG TGGTGGCGAT ACGCGACGCG AGCTCGATTT ATAGGAGGGG 120

ATCGAAGGAG GGGAGCGCGC GCGGCGAGGC CCGCGTTGCT CACCTACGCC GCGCGCATGC 180

GGCGGACGCG CGGTCGGCGC CCGCGCCGGC CGGGAGGACG AGGGCGCAAG CGTGTGAGCC 240

ACCGAACGCG CGCGCGCGCC GCGGCGCGAA CTCTCCATCG CGTCGCGGCG AGCCGAGAGC 300

CGACGAGAGC GTTTCGCGCG CGCGGTTGGG CCGGCGACAA GATGGGCCGT AGCCCTGGGC 360

CTCGTGCCAT CTTTTTTTTT CTTTTTTGCC TTTTTTGGCC TGGCAATTTC TTTTTGTTTT 420

TAGTCTTTTT GTGGTGATAA TGTGTCGTCT TCCGGTGAAC TAATTTACTC GTTGATCTTT 480

TTGTGTCCCT TCGAATATTC GCAGTGGTAG AAGATGACTA CTACTACCAG TAGTTGATCT 540

CGAATGGCAA CTTTTGTGCA GAACTTATTC CACGGCTATG TCAGCTTCCA CTGTGACTAA 600

AAAAACTACG GCCATCTTTT GGACTTGTTC TATCTTGGAA CTGAACAAAA AGGACGATCC 660

TGATGTACAC ACGGCATAGT TTCCAGCACT GGATGCCAAG TTGCCAACTG TTACCACGAT 720

AATGGAACGA CGAGATGAGA TATTATACAA GTCCAATGGA TCAAGATCCT GTGCAGTTGT 780

TATTGTAACT GTAACTTAAG CCGTTAACAT GTACATCACA TTTCCTACTC TATCAATGTC 840

TTGTGCGGGT TGTTTCAAAA AAACATGTAC ATCACATGAT CTAGAACGGA AGGCCAGGAT 900

ATGAAGTGGT ACTGCAGCAA AAACACTGTA GCAGAGATGT ACTATTATGC ATGTACTGTA 960

GCAGTCATCT AGAGCCGTTG GATCTGAAAA CGAATGGACA TGATTGTGTG CAGTTGCTAT 1020

/note= "stamen-specific promoter from corn gene CA55"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:2355..3187
(D) OTHER INFORMATION:/label= P35S
/note= "35S promoter region of Cauliflower mosaic virus"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:3188..3739
(D) OTHER INFORMATION:/label= bar
/note= "region coding for phosphinotricin acetyl transferase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:3757..4017
(D) OTHER INFORMATION:/label= 3'nos
/note= "3' untranslated region containing the polyadenylation
signal of the nopaline synthase gene of Agrobacterium T-DNA"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:699..702
(D) OTHER INFORMATION:/note= "region with unknown
sequence (may contain up to 15 nucleotides)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA 60

CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG 120

TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC 180

ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC 240

ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT 300

TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT 360

TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT CGAGCTCGGT ACCCGGGGAT 420

CTTCCCGATC TAGTAACATA GATGACACCG CGCGCGATAA TTTATCCTAG TTTGCGCGCT 480

ATATTTTGTT TTCTATCGCG TATTAAATGT ATAATTGCGG GACTCTAATC ATAAAAACCC 540

ATCTCATAAA TAACGTCATG CATTACATGT TAATTATTAC ATGCTTAACG TAATTCAACA 600

GAAATTATAT GATAATCATC GCAAGACCGG CAACAGGATT CAATCTTAAG AAACTTTATT 660

CAGGCATGCA AGCTCCTACG CAGCAGGTCT CATCAAGACG ATCTACCCGA GTAACAATCT 2400

CCAGGAGATC AAATACCTTC CCAAGAAGGT TAAAGATGCA GTCAAAAGAT TCAGGACTAA 2460

TTGCATCAAG AACACAGAGA AAGACATATT TCTCAAGATC AGAAGTACTA TTCCAGTATG 2520

GACGATTCAA GGCTTGCTTC ATAAACCAAG GCAAGTAATA GAGATTGGAG TCTCTAAAAA 2580

GGTAGTTCCT ACTGAATCTA AGGCCATGCA TGGAGTCTAA GATTCAAATC GAGGATCTAA 2640

CAGAACTCGC CGTGAAGACT GGCGAACAGT TCATACAGAG TCTTTTACGA CTCAATGACA 2700

AGAAGAAAAT CTTCGTCAAC ATGGTGGAGC ACGACACTCT GGTCTACTCC AAAAATGTCA 2760

AAGATACAGT CTCAGAAGAC CAAAGGGCTA TTGAGACTTT TCAACAAAGG ATAATTTCGG 2820

GAAACCTCCT CGGATTCCAT TGCCCAGCTA TCTGTCACTT CATCGAAAGG ACAGTAGAAA 2880

AGGAAGGTGG CTCCTACAAA TGCCATCATT GCGATAAAGG AAAGGCTATC ATTCAAGATG 2940

CCTCTGCCGA CAGTGGTCCC AAAGATGGAC CCCCACCCAC GAGGAGCATC GTGGAAAAAG 3000

AAGACGTTCC AACCACGTCT TCAAAGCAAG TGGATTGATG TGACATCTCC ACTGACGTAA 3060

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TTCATTTGGA GAGGACACGC TGAAATCACC AGTCTCTCTC TATAAATCTA TCTCTCTCTC 3180

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CTGGCTCGTC GCCGAGGTGG ACGGCGAGGT CGCCGGCATC GCCTACGCGG GCCCCTGGAA 3420

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GCGGACGGGA CTGGGCTCCA CGCTCTACAC CCACCTGCTG AAGTCCCTGG AGGCACAGGG 3540

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GGCGCTCGGA TATGCCCCCC GCGGCATGCT GCGGGCGGCC GGCTTCAAGC ACGGGAACTG 3660

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GCCCGTCACC GAGATCTGAT CTCACGCGTC TAGGATCCGA AGCAGATCGT TCAAACATTT 3780

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TTCTGTTGAA TTACGTTAAG CATGTAATAA TTAACATGTA ATGCATGACG TTATTTATGA 3900

GATGGGTTTT TATGATTAGA GTCCCGCAAT TATACATTTA ATACGCGATA GAAAACAAAA 3960

GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG 5700

TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCCGTAA 5760

GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC 5820

GACCGAGTTG CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT 5880

TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG ATCTTACCGC 5940

TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA 6000

CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA 6060

TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA 6120

TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC 6180

AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCTAA GAAACCATTA 6240

TTATCATGAC ATTAACCTAT AAAAATAGGC GTATCACGAG GCCCTTTCGT C 6291

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T-DNA of plasmid pTHW142

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:1..25
(D) OTHER INFORMATION:/label= RB
/note= "right border sequence of octopine TL-DNA from pTiB6S3"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (84..296)
(D) OTHER INFORMATION:/label= 3'g7
/note= "3' untranslated region containing the polyadenylation signal of gene 7 of Agrobacterium T-DNA"

(ix) FEATURE:

(B) LOCATION:5077..5078
(D) OTHER INFORMATION:/note= "region with unknown sequence (may contain up to 20 nucleotides)"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:5476..5479
(D) OTHER INFORMATION:/note= "region with unknown sequence (may contain up to 20 nucleotides)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AATTACAACG GTATATATCC TGCCAGTACT CGGCCGTCGA GTACATGGTC GATAAGAAAA 60

GGCAATTTGT AGATGTTAAT TCCCATCTTG AAAGAAATAT AGTTTAAATA TTTATTGATA 120

AAATAACAAG TCAGGTATTA TAGTCCAAGC AAAAACATAA ATTTATTGAT GCAAGTTTAA 180

ATTCAGAAAT ATTTCAATAA CTGATTATAT CAGCTGGTAC ATTGCCGTAG ATGAAAGACT 240

GAGTGCGATA TTATGTGTAA TACATAAATT GATGATATAG CTAGCTTAGC TCATCGGGGG 300

ATCCTAGACG CGTGAGATCA GATCTCGGTG ACGGGCAGGA CCGGACGGGG CGGTACCGGC 360

AGGCTGAAGT CCAGCTGCCA GAAACCCACG TCATGCCAGT TCCCGTGCTT GAAGCCGGCC 420

GCCCGCAGCA TGCCGCGGGG GGCATATCCG AGCGCCTCGT GCATGCGCAC GCTCGGGTCG 480

TTGGGCAGCC CGATGACAGC GACCACGCTC TTGAAGCCCT GTGCCTCCAG GGACTTCAGC 540

AGGTGGGTGT AGAGCGTGGA GCCCAGTCCC GTCCGCTGGT GGCGGGGGGA GACGTACACG 600

GTCGACTCGG CCGTCCAGTC GTAGGCGTTG CGTGCCTTCC AGGGGCCCGC GTAGGCGATG 660

CCGGCGACCT CGCCGTCCAC CTCGGCGACG AGCCAGGGAT AGCGCTCCCG CAGACGGACG 720

AGGTCGTCCG TCCACTCCTG CGGTTCCTGC GGCTCGGTAC GGAAGTTGAC CGTGCTTGTC 780

TCGATGTAGT GGTTGACGAT GGTGCAGACC GCCGGCATGT CCGCCTCGGT GGCACGGCGG 840

ATGTCGGCCG GGCGTCGTTC TGGGTCCATG CAGTTAACTC TTCCGCCGTT GCTTGTGATG 900

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GAAAAATAGA GAGAGATAGA TTTGTAGAGA GAGACTGGTG ATTTTTGCGC CGGGTACCGA 3000

GCTCGGTAGC AATTCCCGAG GCTGTAGCCG ACGATGGTGC GCCAGGAGAG TTGTTGATTC 3060

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GCAGAAAAGC CGCCGACTTC GGTTTGCGGT CGCGAGTGAA GATCCCTTTC TTGTTACCGC 3180

CAACGCGCAA TATGCCTTGC GAGGTCGCAA AATCGGCGAA ATTCCATACC TGTTCACCGA 3240

CGACGGCGCT GACGCGATCA AAGACGCGGT GATACATATC CAGCCATGCA CACTGATACT 3300

CTTCACTCCA CATGTCGGTG TACATTGAGT GCAGCCCGGC TAACGTATCC ACGCCGTATT 3360

CGGTGATGAT AATCGGCTGA TGCAGTTTCT CCTGCCAGGC CAGAAGTTCT TTTTCCAGTA 3420

CCTTCTCTGC CGTTTCCAAA TCGCCGCTTT GGACATACCA TCCGTAATAA CGGTTCAGGC 3480

ACAGCACATC AAAGAGATCG CTGATGGTAT CGGTGTGAGC GTCGCAGAAC ATTACATTGA 3540

CGCAGGTGAT CGGACGCGTC GGGTCGAGTT TACGCGTTGC TTCCGCCAGT GGCGAAATAT 3600

TCCCGTGCAC TTGCGGACGG GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG 3660

GGTGGTTTTT GTCACGCGCT ATCAGCTCTT TAATCGCCTG TAAGTGCGCT TGCTGAGTTT 3720

CCCCGTTGAC TGCCTCTTCG CTGTACAGTT CTTTCGGCTT GTTGCCCGCT TCGAAACCAA 3780

TGCCTAAAGA GAGGTTAAAG CCGACAGCAG CAGTTTCATC AATCACCACG ATGCCATGTT 3840

CATCTGCCCA GTCGAGCATC TCTTCAGCGT AAGGGTAATG CGAGGTACGG TAGGAGTTGG 3900

CCCCAATCCA GTCCATTAAT GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC 3960

CACGTAAGTC CGCATCTTCA TGACGACCAA AGCCAGTAAA GTAGAACGGT TTGTGGTTAA 4020

TCAGGAACTG TTCGCCCTTC ACTGCCACTG ACCGGATGCC GACGCGAAGC GGGTAGATAT 4080

CACACTCTGT CTGGCTTTTG GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT 4140

GCCAGAGGTG CGGATTCACC ACTTGCAAAG TCCCGCTAGT GCCTTGTCCA GTTGCAACCA 4200

CCTGTTGATC CGCATCACGC AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT 4260

CAACAGACGC GTGGTTACAG TCTTGCGCGA CATGCGTCAC CACGGTGATA TCGTCCACCC 4320

AGGTGTTCGG CGTGGTGTAG AGCATTACGC TGCGATGGAT TCCGGCATAG TTAAAGAAAT 4380

CATGGAAGTA AGACTGCTTT TTCTTGCCGT TTTCGTCGGT AATCACCATT CCCGGCGGGA 4440

TAGTCTGCCA GTTCAGTTCG TTGTTCACAC AAACGGTGAT ACCTGCACAT CACCATGTTT 4500

TGGTCATATA TTAGAAAAGT TATAAATTAA AATATACACA CTTATAAACT ACAGAAAAGC 4560

CLAIMS

1. A process for producing transgenic eucaryote cells which comprises:
contacting a culture of untransformed cells with an inhibitor of poly-(ADP-ribose) polymerase, prior to transformation, for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce the metabolism of said cultured cells, particularly to reduce the electron flow in the mitochondrial electron transport chain; contacting said untransformed cells with foreign DNA comprising at least one gene of interest under conditions in which said foreign DNA is taken up by said untransformed cells and said gene of interest is stably integrated in the nuclear genome of said untransformed cells to produce said transgenic cells; and
optionally recovering said transgenic cells from said culture.

2. The proces of claim 1, wherein said eucaryotic cells are plant cells.

3. The process of claim 1 or 2, wherein said inhibitor is niacinamide, preferably at a concentration of about 150 mg/l to 1000 mg/l, more preferably at a concentration of about 200 mg/l to 500 mg/l, particularly at a concentration of about 250 mg/l.

4.The process of any one of claims 1 to 3, wherein said untransformed cells are cultured in a medium containing said inhibitor for a period of time of approximately 2 to 28 days, preferably approximately 3 to 14 days, particularly approximately 4 days prior to the contacting with said foreign DNA.

5. The process of any one of claims 1 to 4, wherein said cells contacted with said foreign DNA are further cultured in a medium containing said inhibitor

11. The process of any one of claims 1 to 10, wherein a transgenic organism having said foreign DNA with said at least one gene of interest stably integrated in its genome is obtained from said transformed eucaryotic cell.

12. The proces of claim 11, wherein said organism is a plant which is obtained by regeneration from a transformed plant cell.

13. The transgenic organism obtained by the process of claim 11 or 12.

14. A plant having foreign DNA integrated in the nuclear DNA of its cells only in the regions of said nuclear DNA that are transcriptionally active in said cells of said plant when said cells are treated with an effective amount of a PARP inhibitor for a period of time sufficient to reduce cell metabolism to a state where gene expression is essentially limited to genes expressed irrespective of the differentiated or physiological condition of the cell.

15. The plant according to claim 14, wherein said integration of the foreign DNA in said transcriptionally active region is verified by measuring the level of expressed mRNA corresponding to this foreign DNA when said cells are incubated in a medium containing a PARP-inhibitor.

16. The plant according to claim 14, wherein said transcriptionally active regions of the genome of said plant include regions which are minimally affected by cell differentiation or cell physiological and biochemical changes caused by external factors such as environmantal conditions, especially stress conditions.

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(57) Abstract

The invention concerns a process for producing transgenic eucaryotic cells, particularly plants, which comprises: contacting a culture of untransformed cells with an inhibitor of poly-(ADP-ribose) polymerase for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce their metabolism. The untransformed cells are then contacted with foreign DNA comprising at least one gene of interest under conditions in which the foreign DNA is taken up by the untransformed cells and the gene of interest is stably integrated in the nuclear genome of the untransformed cells to produce the transgenic cells. Optionally, the transgenic cells are recovered from the culture. Preferably, the inhibitor is niacinamide, preferably at a concentration of about 200 mg/l to 500 mg/l and the untransformed cells are cultured in a medium containing the inhibitor for a period of time of approximately 3 to 14 days prior to the contacting with the foreign DNA. The invention also relates to a plant having in the nuclear genome of its cells foreign DNA integrated only in the regions of the nuclear genome that are transcriptionally active in cells of the plant when the cells are treated with an effective amount of a PARP inhibitor for a period of time sufficient to reduce cell metabolism to a state where gene expression is essentially limited to genes expressed irrespective of the differentiated or physiological condition of the cell.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUCLEIC ACIDS RES 18 (20). 1990. 5981-5988., XP002023138 WALDMAN B C: " ILLEGITIMATE AND HOMOLOGOUS RECOMBINATION IN MAMMALIAN CELLS DIFFERENTIAL SENSITIVITY TO AN INHIBITOR OF POLY-ADP-RIBOSYLATION." cited in the application see the whole document ---	1,23
A	EP,A,0 424 047 (PIONEER HI BRED INT) 24 April 1991 see page 2, line 32 - page 3, line 9 --- -/--	6

[X] Further documents are listed in the continuation of box C.

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PCT/EP 96/03366

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COMPTES RENDUS DE L'ACADEMIE DES SCIENCES SERIE III SCIENCES DE LA VIE 318 (1). 1995. 121-128., XP002023143 DEVIC M., ET AL.: " Assessment of promoter trap as a tool to study zygotic embryogenesis in Arabidopsis thaliana." see page 124, right-hand column ---	14,23
A	CHEMICAL ABSTRACTS, vol. 123, no. 21, 20 November 1995 Columbus, Ohio, US; abstract no. 276968, MANDAL, ABUL: "Identification of Arabidopsis thaliana sequences responsive to low temperature and abscisic acid by T-DNA tagging and in-vivo gen fusion" XP002023145 see abstract & PLANT MOL. BIOL. REP. (1995), VOLUME DATE 1995, 13(3), 243-54, -----	14,23